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Short sequence-paper

Sequence and expression of a cDNA encoding the red seabream androgen receptor[1]

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Abstract

The cDNA of the androgen receptor (AR) has been isolated from the ovary of red seabream, *Pagrus major*, and sequenced. The amino acid sequence of red seabream AR (rsAR) shows about 45% identity with that of *Xenopus*, rat, mouse, and human ARS. It is shown that rsAR has the ability to *trans*-activate the responsive gene depending on the presence of androgen.

Keywords: Androgen; Androgen receptor; *Trans*-activation function; 11-Ketotestosterone; Teleost

In all vertebrates, androgen is defined as a hormone which is involved in masculine traits. In a teleost fish as in other vertebrates, androgen mainly controls reproductive behaviour and spermatogenesis [1]. These actions require binding of androgen to a specific receptor protein, androgen receptor (AR). The resultant complex represents a *trans*-activation function that stimulates the transcription by binding to a specific DNA element termed a hormone-responsive element [2]. Among higher vertebrates, AR cDNA clones of human [3–6], rat [4], mouse [7], and *Xenopus* [8] have been isolated and sequenced. AR belongs to the family of steroid hormone receptors [3,4,6]. In mammals, major androgens are testosterone and 5α-dihydrotestosterone. In teleost fish, 11-ketotestosterone also has an androgenous function and is known to be more effective than testosterone in stimulating secondary male characters, reproductive behavior, and spermatogenesis [1]. Thus 11-ketotestosterone is considered to be a major androgen in male teleost; however, a receptor for 11-ketotestosterone or testosterone has not yet been identified.

In order to isolate red seabream (*Pagrus major*) AR (rsAR) cDNA clone, we designed a set of oligonucleotide mixed primers, (5′-d(CGGGATCC-GA(A,G)GG(A,C,G,T)AA(A,G)CA(A,G)AA(A,G)-TA)) and (5′-d(ATGGATCC(A,C,G,T)GC(C,T)TT-CAT(A,C,G,T)A(A,G)(A,G)AA(C,T)TC)) (Fig. 1), based on an amino acid sequence which is highly conserved among mammalian ARs. Transcription into cDNA and the reverse transcriptase–polymerase chain reaction (RT–PCR) using above primers were performed as described previously [9]. After 30 cycles

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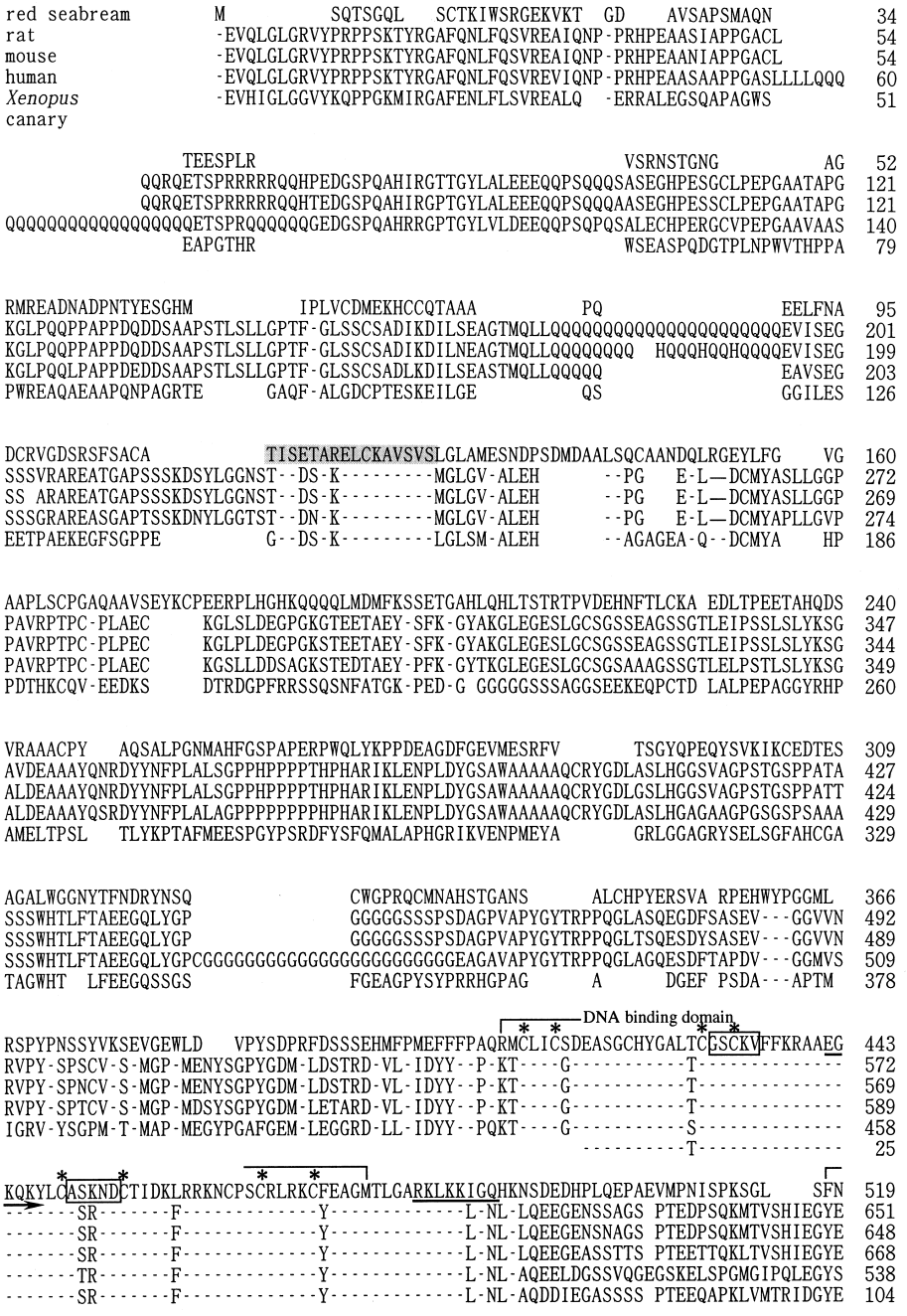

Fig. 1. Comparison of the deduced amino acid sequences of red seabream, rat, mouse, human, *Xenopus* and canary androgen receptors. The numbers on the right are the positions of the amino acids. Identical amino acids to those rsAR are indicated by dashes (−) in the other ARs. Gaps are introduced to maximize the alignment and are indicated by spaces. The former and latter open box represent the P-box and D-box, respectively. The arrows, underlining, and asterisks represent the mixed primers used in PCR, the sequence homologous to nuclear localization signal, and cysteine residues in zinc-finger, respectively. The shadowed box represents highly conserved region of N-terminal domain between red seabream and mammalian ARs.

(0.5 min at 94°C, 1 min at 50°C, and 1.5 min at 72°C) of PCR, the product was separated by electrophoresis. The product showed the expected size, and it was subcloned and sequenced. Its sequence showed a high homology to canary (GenBank accession no. L25901) and mouse (GenBank accession no.

M37890) ARs by the homology search. The cDNA library of red sea bream ovary was synthesized with a λZAPII-cDNA Synthesis kit (Stratagene). The phages were plated onto *Escherichia coli*. XLI-Blue and then transferred to nylon membranes in duplicate. About 6.5×10^5 plaques from the cDNA library of red sea bream ovary were screened with the above RT–PCR product which was labeled with ^{32}P using a Megaprime DNA labeling system (Amersham), and two positive clones were obtained. One clone of rsAR had a complete open reading frame (ORF) of 769 amino acid residues and the other clone lacked 5′ end of the ORF of the same gene. The DNA sequence surrounding the ATG codon showed good agreement with the consensus sequence proposed by Kozak [10] for the translation initiation region (GNNATGN).

The amino acid sequence of rsAR showed 43%, 46%, 45%, and 44% identity with those of *Xenopus*, rat, mouse, and human ARs, respectively (Fig. 1). Structural analyses of cDNAs for various steroid hormone receptors have revealed the functional domains responsible for transcriptional activation, DNA binding, nuclear localization, and hormone binding. The amino acid sequence of the DNA-binding domain of rsAR showed 89% and 92% identity with those of *Xenopus* and mammalian ARs, respectively. In this domain, eight cysteine residues in two zinc-fingers and the P-box, both of which are important for binding to and recognition of the hormone-responsive element [11], are completely conserved among all ARs (Fig. 1). On the other hand, in rsAR, lysine residue of the D-box, which is important for the determination of the half-site spacing [11], was substituted for arginine compared with mammalian ARs. The amino acid sequence of hormone-binding domain is also conserved among various species, since 72% and 71% of identity in the amino acid sequence is retained with *Xenopus* and mammalian ARs, respectively (Fig. 1). A putative nuclear localization signal sequence as found in other steroid hormone receptor [12], homologous to that of the SV40 large T-antigen, was located at the position of 10 amino acids after the last conserved cysteine (Fig. 1).

In contrast, nonhomologous structures were found in the N-terminal region between rsAR and mammalian ARs (Fig. 1). The N-terminal region of rsAR

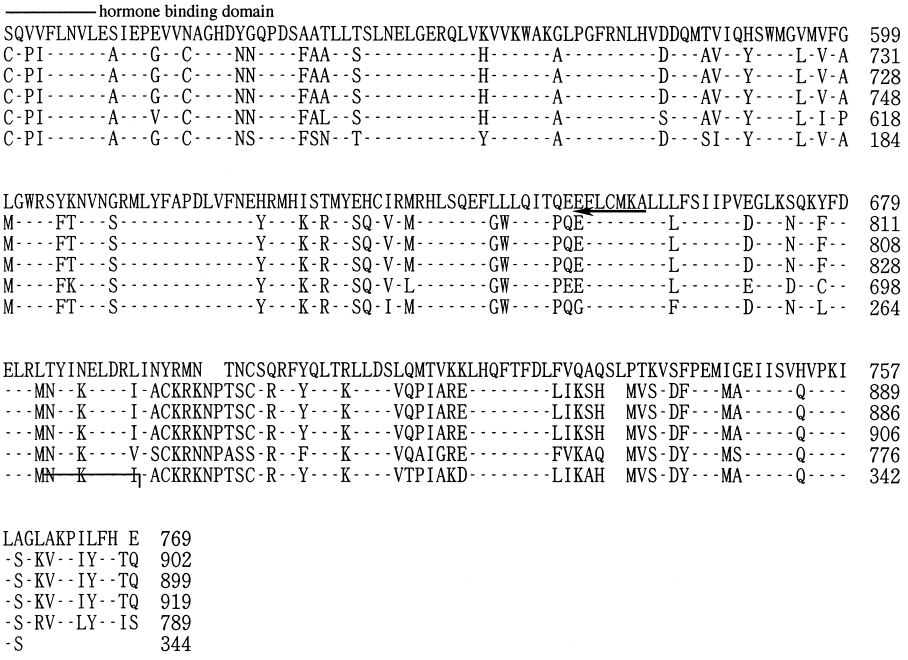

Fig. 2. Transcriptional activity of red sea bream androgen receptor in COS-7 cells. COS-7 cells were cotransfected with either pcDNA3 or pcDNA3-rsAR and pMSG-CAT. The results are expressed as the relative CAT activity averaged for duplicate determinations after correction for the internal control in the presence (open box) or absence (shadowed box) of 11-ketotestosterone or testosterone.

was shorter than those of rat, mouse, and human ARs. This is due to the lack of poly-glutamine or poly-glycine, which are present in N-terminal region of mammalian ARs. The N-terminal region of rsAR showed 18%, 25%, 22%, and 22% identity of amino acid residues with those of *Xenopus*, rat, mouse, and human ARs, respectively (Fig. 1). In spite of these nonhomologous structures, the rsAR cDNA had *trans*-activation function in mammalian cells as described below. The N-terminal region is considered to be important in species- or tissue-specific functions and/or modulation of receptor activity [13,14]. Despite the low overall homology in the N-terminal region, the amino acid residues located from threonine 110 to leucine 128 of rsAR are conserved with the corresponding region of mammalian ARs (Fig. 1). This region is rich in serine and threonine residues, which are putatively phosphorylated by the various protein kinases. In fact, it was reported that the N-terminal region of the estrogen receptor contained the conserved specific sequence phosphorylated by a mitogen-activated protein kinase, which was important for the full activity of the *trans*-activation function [15].

The *trans*-activation function of rsAR was verified by means of a transient expression assay. The expression vector of rsAR, pcDNA3-rsAR, was constructed by subcloning rsAR into a CMV-based expression vector, pcDNA3 (Stratagene). The chimeric reporter plasmid, pMSG-CAT, consisting of the androgen-responsive MMTV promoter [2] and the chloramphenicol acetyl transferase (CAT) gene was purchased from Pharmacia. pcDNA3-rsAR and pMSG-CAT were cotransfected into COS-7 cells by using a Gene Pulsar II (Bio-Rad), and CAT assay was performed in the presence of glucocorticoid, androgen, estrogen, and progesterone-like steroid hormone, as described previously [9]. While no transactivation activity was observed in the presence of $1.0-10^{-6}$ M dexamethasone, estradiol-17β, and 17α, 20β-dihydroxy-4-pregnen-3-one, the CAT activity was exclusively stimulated in the presence of 11-ketotestosterone (data not shown). Although the rsAR is the least conserved among ARs reported, these results indicate that rsAR has the ability to *trans*-activate the responsive gene in the presence of androgen. Since 11-ketotestosterone is known to be more effective than testosterone in stimulating secondary male characters, reproductive behavior, and spermatogenesis in teleost fish [1], effects of testosterone and 11-ketotestosterone on the *trans*-activating activity of rsAR were compared by cotransfection pcDNA3-rsAR and pMSG-CAT into COS-7 at the concentration of 1.0×10^{-6} M (Fig. 2). Cotransfection of pcDNA3 and pMSG-CAT resulted in low activity of CAT in the presence or absence of testosterone. In contrast, testosterone and 11-ketotestosterone stimulated the CAT activity by up to 24- and 26-fold, respectively, compared with the absence of androgen in cells cotransfected with pcDNA3-rsAR and pMSG-CAT (Fig. 2). This result suggested that there is no difference in the ability to stimulate transcription between 11-ketotestosterone and testosterone in this transient expression assay.

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